Apath to discovery
www.apath.com

DMID/NIAID/NIH Optimizing Positive "Hits" for Potency and Safety in Anti-Infective Drug Development Feb 7-8, 2007

Identification of inhibitors of Ebola virus using a subgenomic replication system.

Paul D. Olivo, M.D., Ph.D.
President & Chief Scientific Officer
Apath, LLC

Apath

- Apath is an early stage drug discovery company focused on antivirals
- Discovery platform is well suited to bioterrorism agents

Apath DD Strategy

- **Screening platform based on subgenomic replication systems (replicons)**
  - **10 viruses (4 biodefense pathogens)**
- **Focused on preclinical studies**
  - **Commercial collaborators and service contracts for chemistry, pharmacokinetic, and toxicology studies**
  - **Academic and government collaborators for MOA studies**
- **Seeking partners for clinical development**

Viral hemorrhagic fever

- **Arenaviruses**
  - **Argentine, Bolivian, Venezualan HF**
  - **Lassa fever**
- **Bunyaviruses**
  - **Congo-Crimean, Rift Valley, Hantaan**
- **Flaviviruses**
  - **Yellow fever, Dengue**
- **Filoviruses**
  - **Ebola, Marburg**

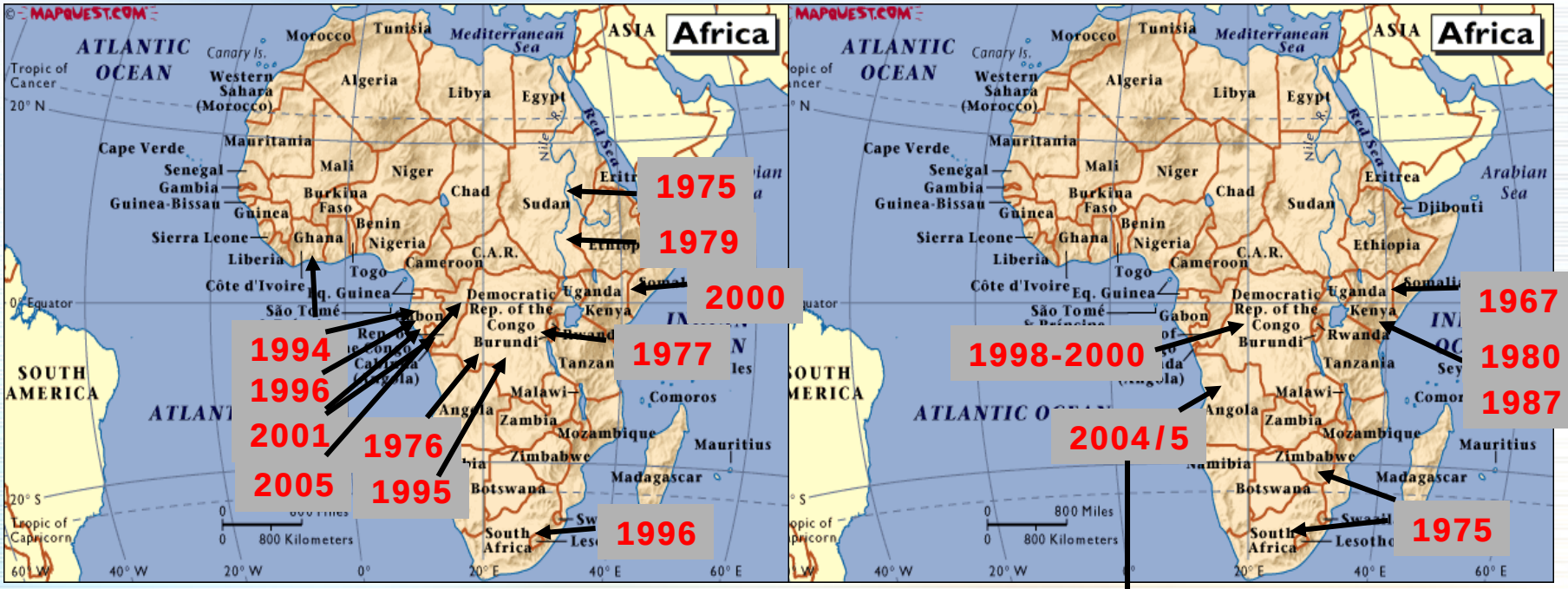
Filovirus Viral Hemorrhagic Fever

- **Ebola and Marburg viruses**
  - Ebola Subtypes: Zaire, Sudan, Ivory Coast, Gabon, Reston
- **Epidemiology**
  - Natural host is unknown (bats?)
  - Transmission associated with contact with body fluids
- **Clinical Features**
  - Incubation period: 4-21 days
  - Abrupt onset of nonspecific symptoms
  - Impaired liver function
  - Dysregulated coagulation/bleeding diathesis
  - Severe morbidity/shock 6-9 days after onset
  - High case-fatality rate (40-90%)
  - No vaccine or effective antiviral therapy

Filovirus hemorrhagic fever

Ebola outbreaks

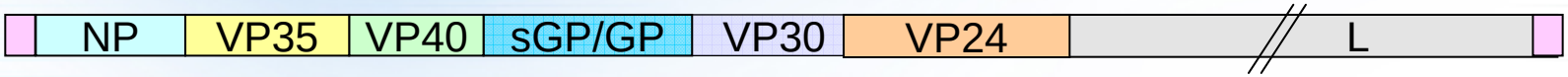
Marburg outbreaks



422 cases (356 deaths; >80% case-fatality)

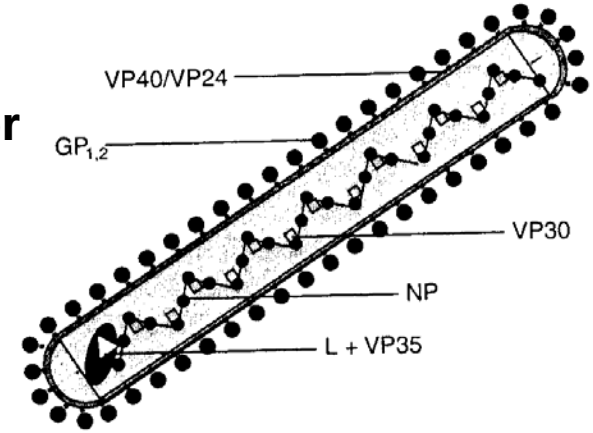
Ebola virus

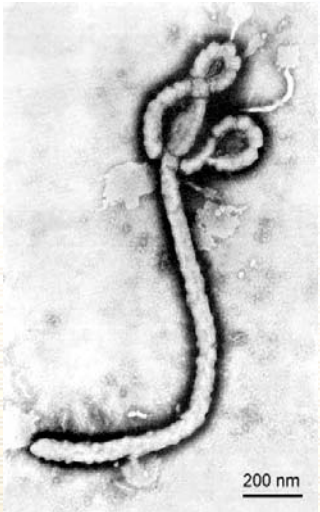
- **family: Filoviridae (filo (latin): 'threadlike')**
- **enveloped**
- **genome: negative-sense, single-stranded RNA, 19 kb**



Viral Proteins:

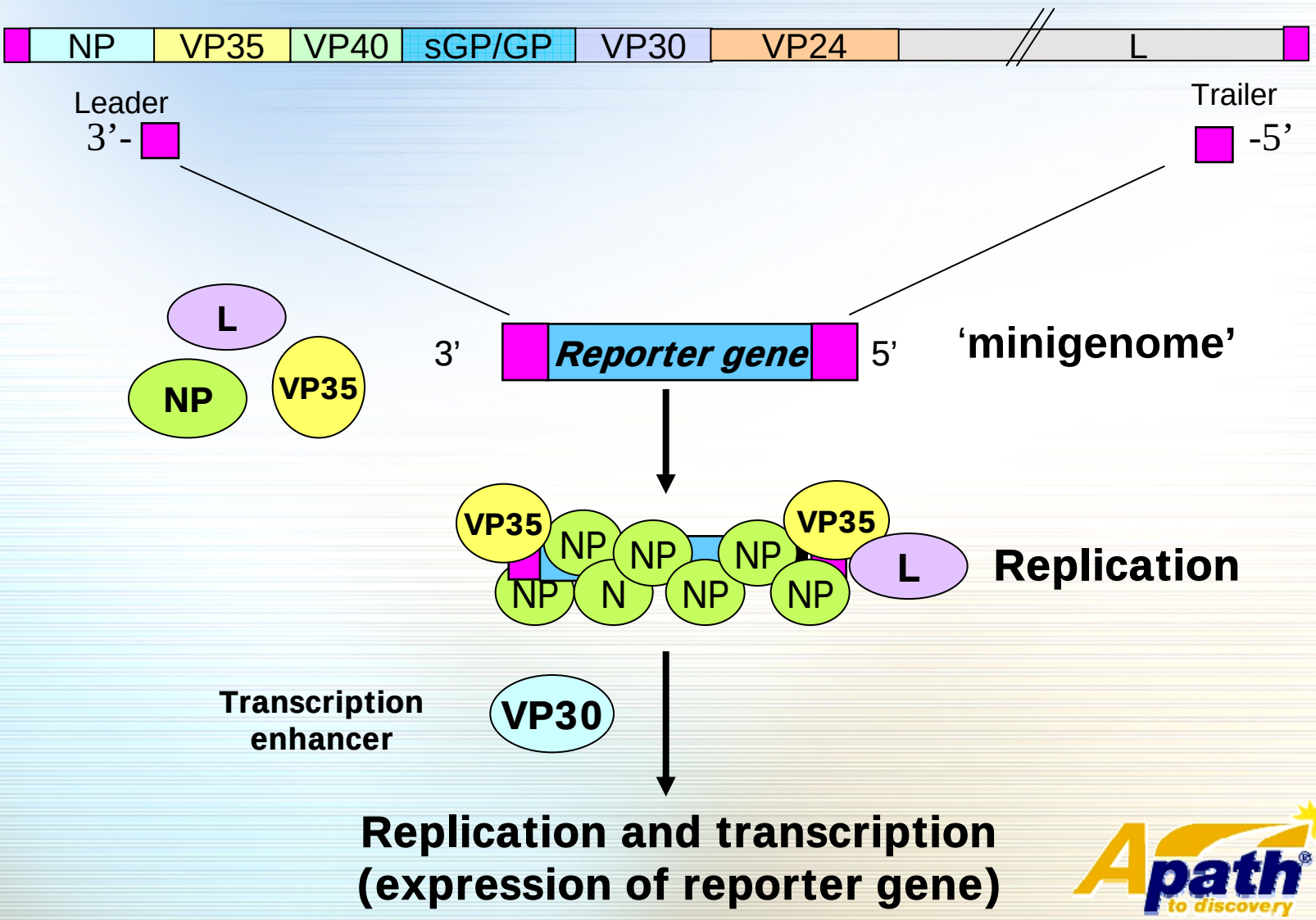
L = polymerase
VP35 = polymerase cofactor
NP = nucleoprotein
VP30 = transcription factor
GP = glycoprotein
VP40 = matrix protein
VP24 = matrix protein





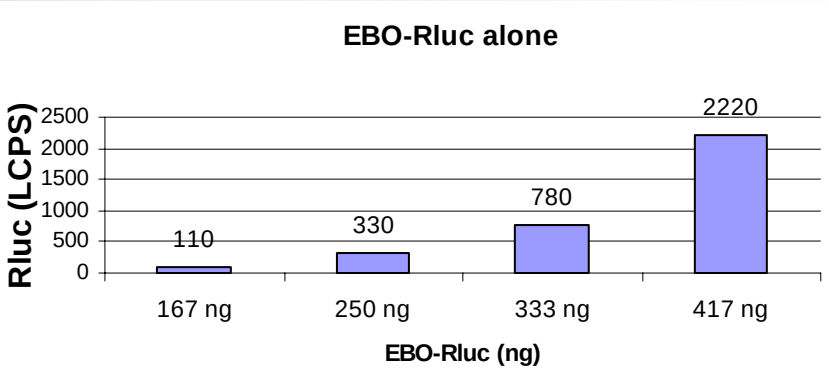
EM image
Frederick A. Murphy, CDC

EBOV subgenomic replication



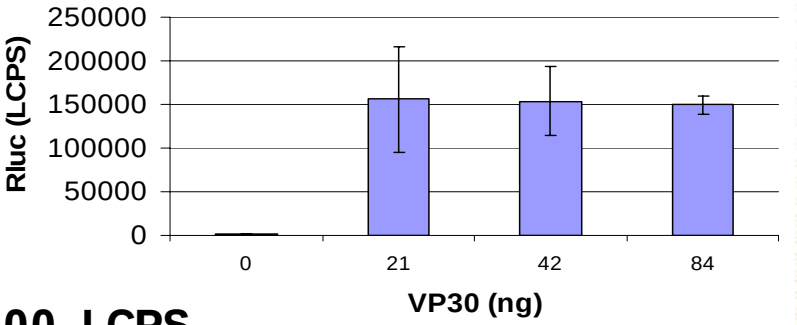
Reporter gene expression is dependent on viral proteins

Minigenome with reporter gene



T7 pol expression vectors for:

- **NP**
- **VP35**
- **L**
- **VP30**



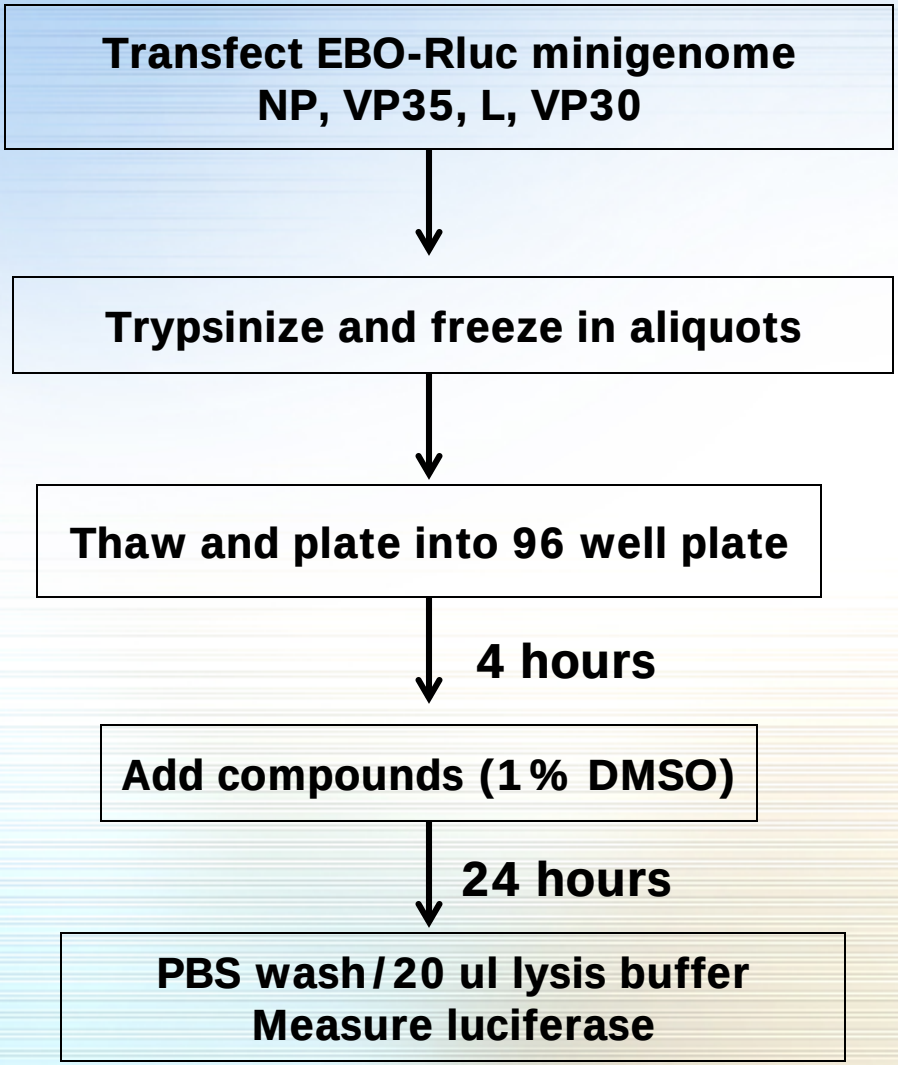
Signal: ca 150000 LCPS
Noise: ca 100 LCPS
S/N: 1500

EBO-Rluc:	**167 ng**
NP:	**208 ng**
VP35:	**208 ng**
L:	**208 ng**

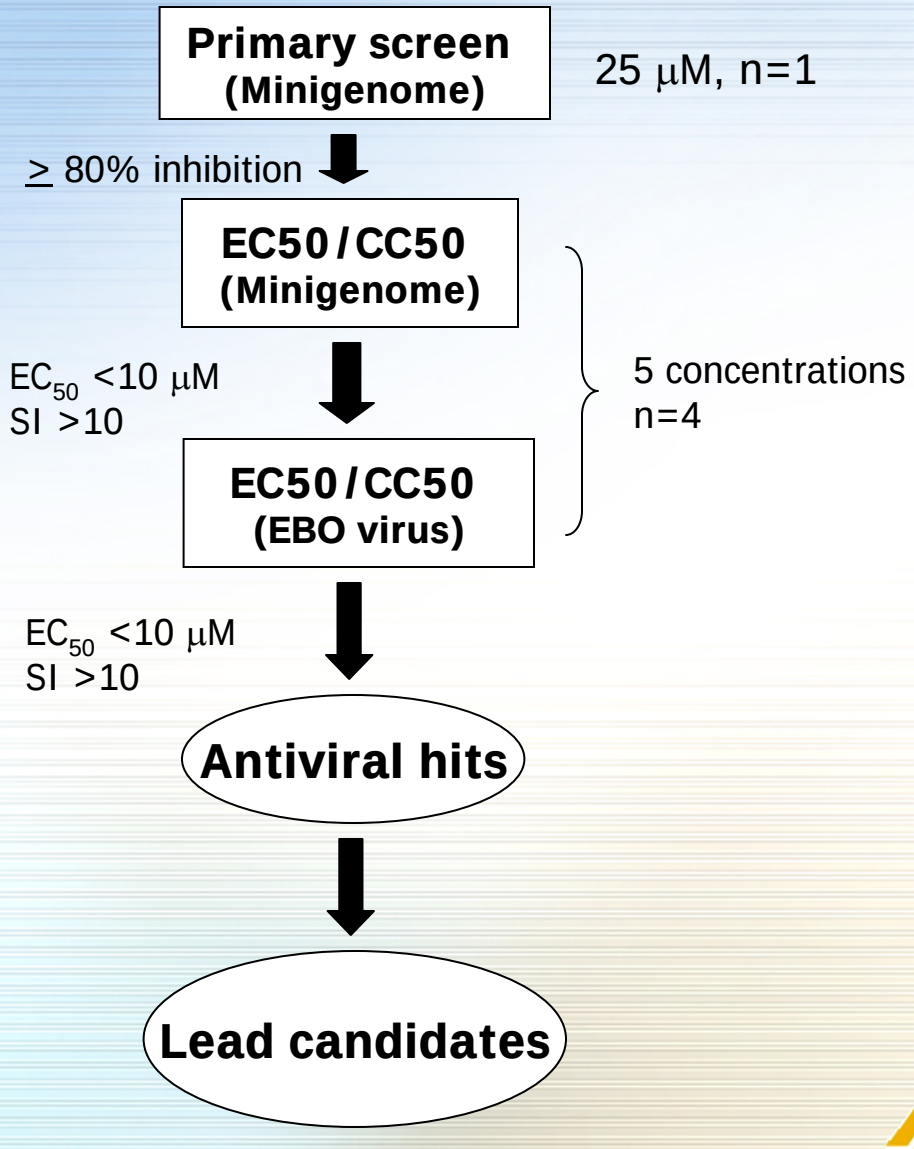
Rationale for minigenome-based screen

- Cell-based assay that can be carried out at BSL-2
- Focus on viral RNA replication, transcription and translation of viral proteins
- Enables the identification of novel targets (viral and host)

EBO-Rluc screening setup



Screening protocol



Ebola viral assays

USMARIID

Cooperative research and development agreement (CRADA)

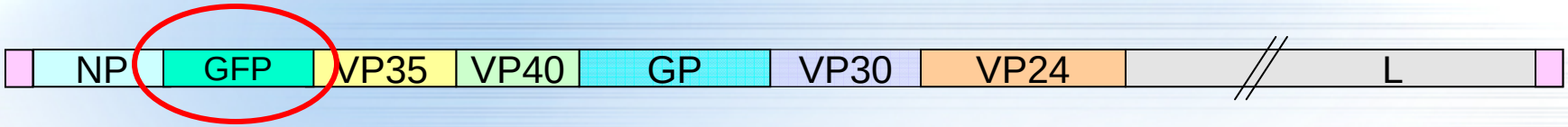
Cell culture assays:

- EBO-GFP infection assay
- CPE inhibition assay
- Plaque reduction assay

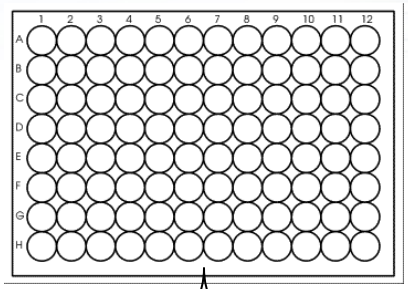
Animal models:

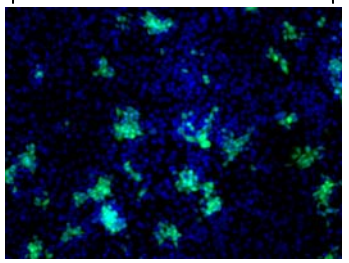
- Mouse model: BALB/c
  - Mouse-adapted strain of Zaire strain
- Monkey model: Rhesus, cynomolgus macaques
  - Zaire/95 strain

Ebola-GFP recombinant virus



- **Zaire strain of Ebola virus**
- **High level of GFP expression**
- **not cytolytic**





Ebola-GFP infection assay:

- Vero E6 cells in 96 plates
- Infection (MOI = 0.1)
- IFN a control (IC90)
- 48h incubation
- Formalin fixation

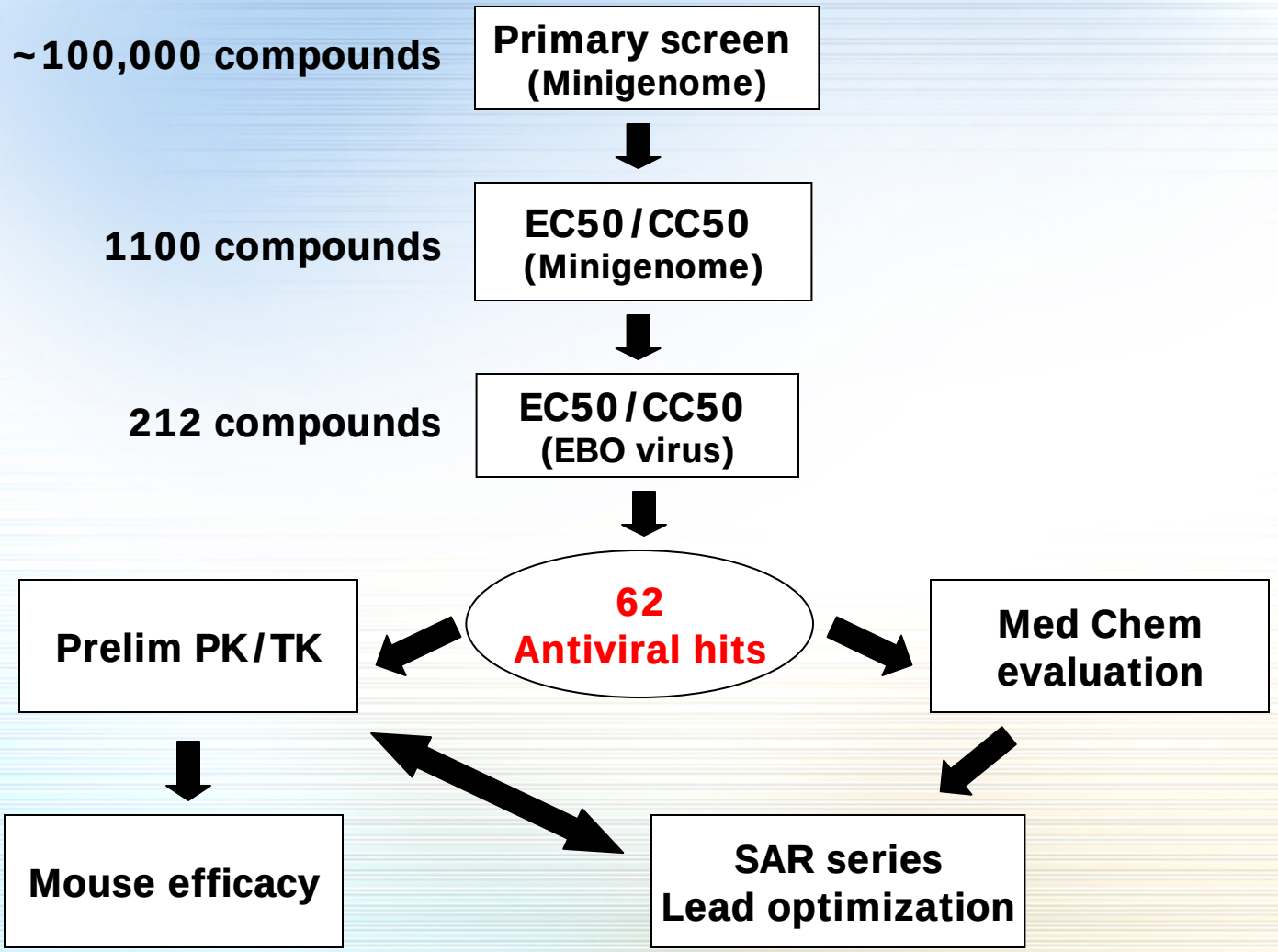
} BCL-4

- Wash out formalin with PBS and soak in PBS (1h)

- GFP detection: Spectrofluorometer (bottom read)
- Signal to noise: S/N = $\geq$12
- Cytotoxicity: crystal violet staining (CC50 @ Apath by ATP-content)

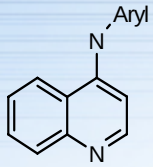
Towner et al. *Virology:* 332(1):20-7; Feb. 5, 2005

Overview

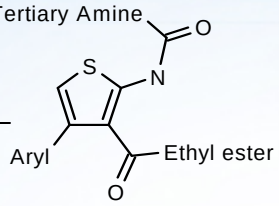


Nine classes of hits

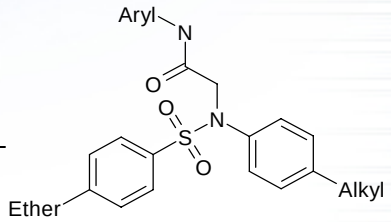
1. **4-Aminoquinolines**



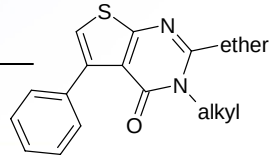
2. **Aminothiophenes**



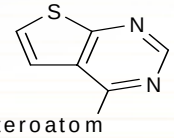
3. **Glycine Tertiary Sulfonamides**



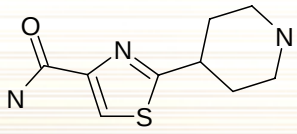
4. **Phenyl thienylpyrimidones**



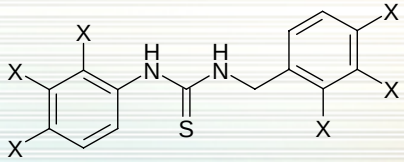
5. **Thienylpyrimidines**



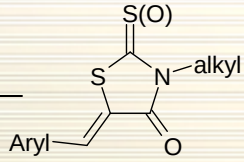
6. **Nipecotic thiazoles**



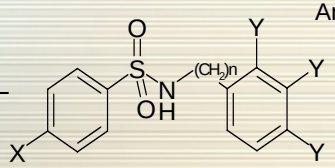
7. **Thioureas**



8. **Rhodanines**



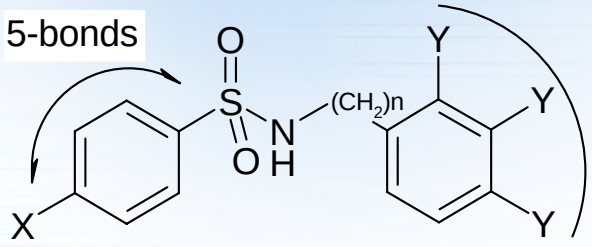
9. **Secondary Sulfonamides**



Rationale for 2^0 sulfonamide SAR series

- Several thousand 2^0 sulfonamides in library (includes Tripos LeadQuest™) which allowed for a preliminary SAR analysis
- Tripos has proprietary high throughput synthetic methods for 2^0 sulfonamides
- Leverage our efforts with the RSV SAR optimization program by making series in parallel to maximize resources (chemist time/chemical reagents)

Secondary Sulfonamides Lead Candidates



e- withdrawing or hydrophobic groups

- 273 with EC50/CC50 data
- 64 minigenome hits
- **21 Lead candidates**

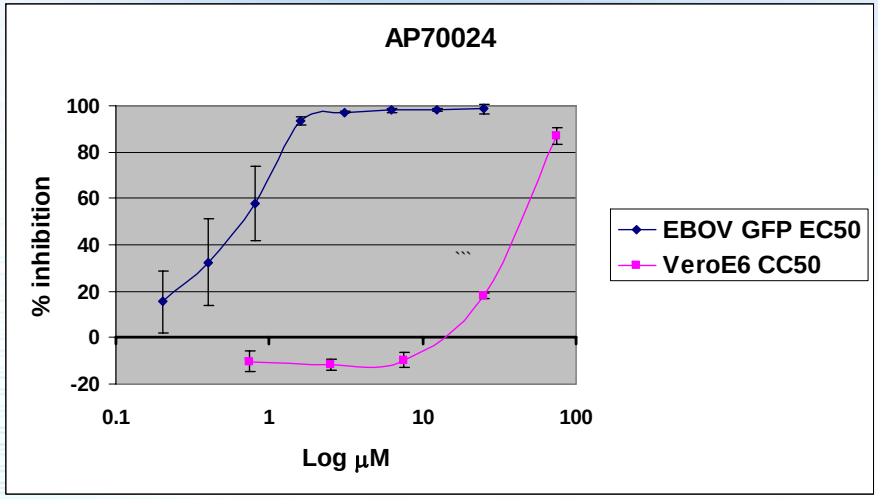
SAR information from HTS:

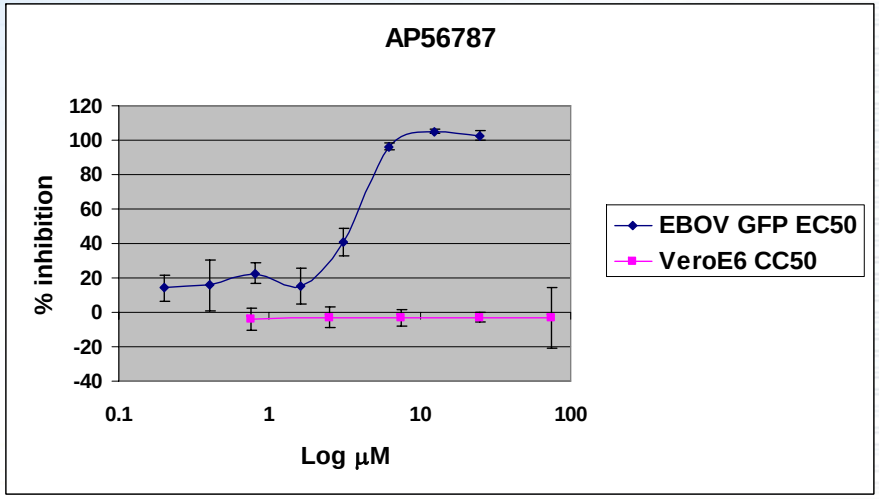
Active structures feature:

- aryl amines substituted with e- withdrawing and hydrophobic substituents
- aryl sulfonic acids heteroatom substitutions 5 bonds from the sulfur

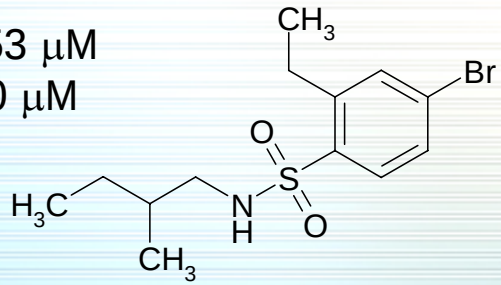
Chemistry – amenable to SAR optimization

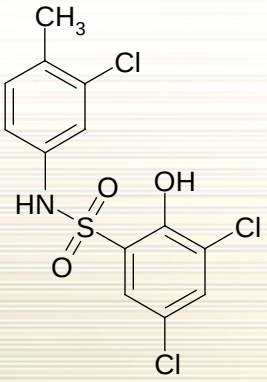
2^0 Sulfonamide lead candidates





EC50 0.53 μM
CC50: 40 μM

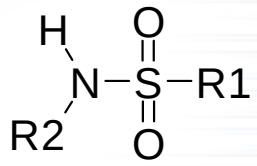




EC50 2.8 μM
CC50: >75 μM

Strategies for Design of SAR Series

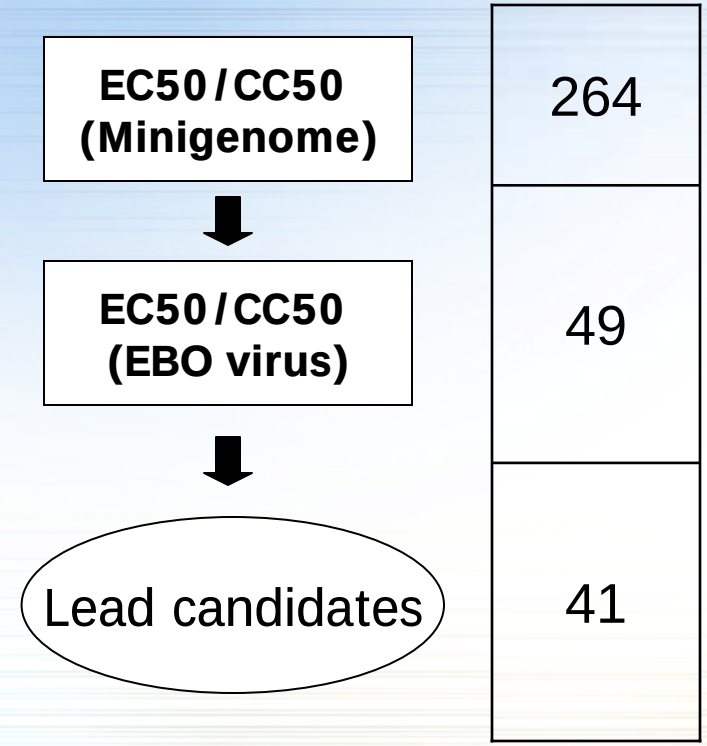
- Strategy 1
  - Perform sidechain analysis of lead candidates and structurally similar compounds (via SAR-Navigator analysis from TRIPOS)
  - Combine R1 and R2 groups from lead candidates



- Strategy 2
  - Create topomeric conformer (topCoMFA, Tripos) model of lead candidates and their neighbors
  - Use topCoMFA model and topomer searching to select reagents for generation potential products

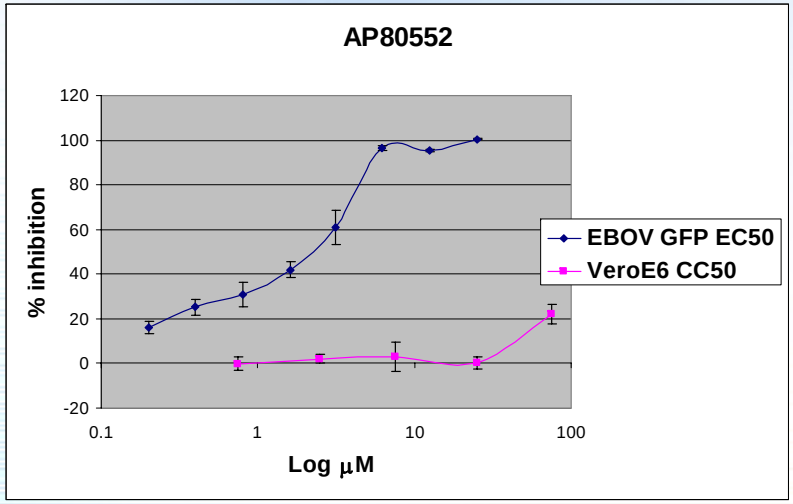
Combined both approaches for the 1st round SAR series (264 compounds)

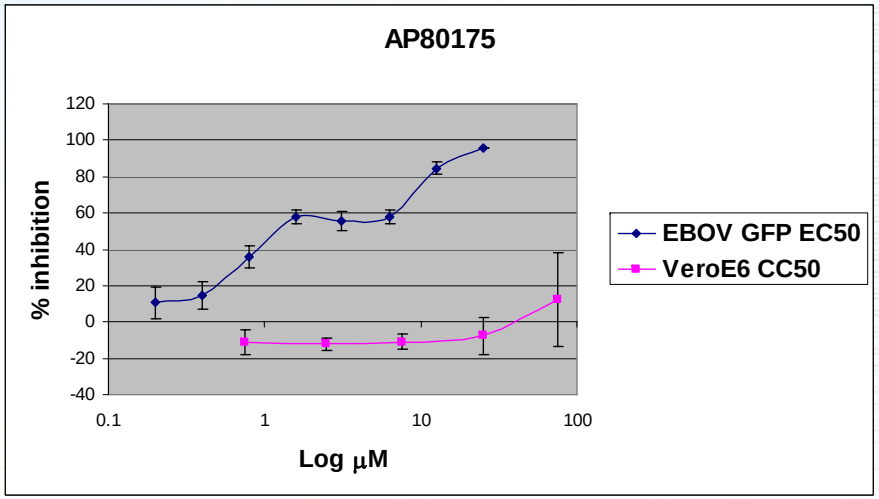
1st round 2⁰ sulfonamide series



- Enrichment in the number of compounds and quality of viral hits (higher SI) when comparing to HTS results
- No improvement of potency in viral GFP EBOV assay!

Novel 2^0 Sulfonamide lead candidates





EC50 3.4 μM
CC50: >75 μM

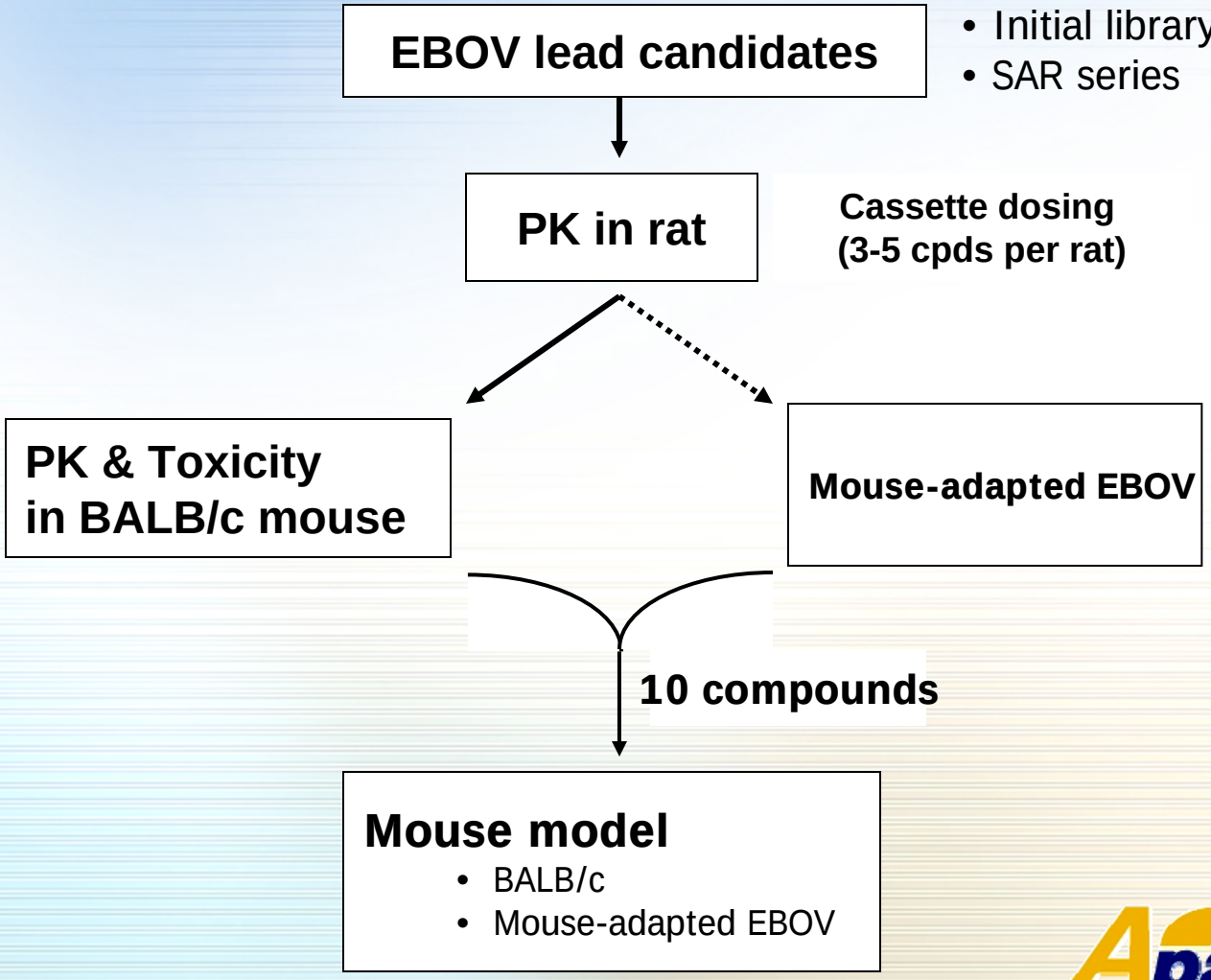
EC50 1.8 μM
CC50: >75 μM

Ebola murine model

- BALB/c mice: 5-16 weeks (female)
- Mouse-adapted virus (Zaire):
  - LD50 = 0.03 pfu (= 1 virion by EM)
  - Death by day 7-8
- Route of infection: IP
- Readout:
  - Weight
  - Survival
  - Viral load (serum, 3-4 log reduction is significant)
- Pathology
  - Histopathology in multiple organs (liver, spleen, LN, Adrenal glands)
  - High titers of virus in liver and spleen
  - EBOV Ag in kidney, lung, GI tract
- Sensitive to drugs that induce type I IFN response
- Good for testing inhibitors of replication.

Bray et al. J Infect Dis. 1999 Feb;179 Suppl 1:S248-58.
Bray et al. J Infect Dis 1998 Nov;178(5):1553.

Selection of compounds for mouse efficacy study



Compounds selected for EBOV efficacy study

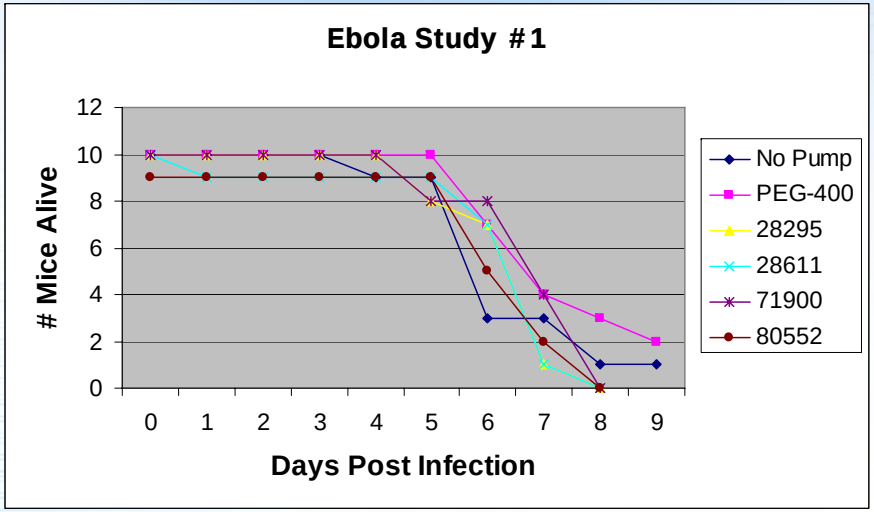
Single IV and IP dosed in Female Balb C Mice at 5 mpk

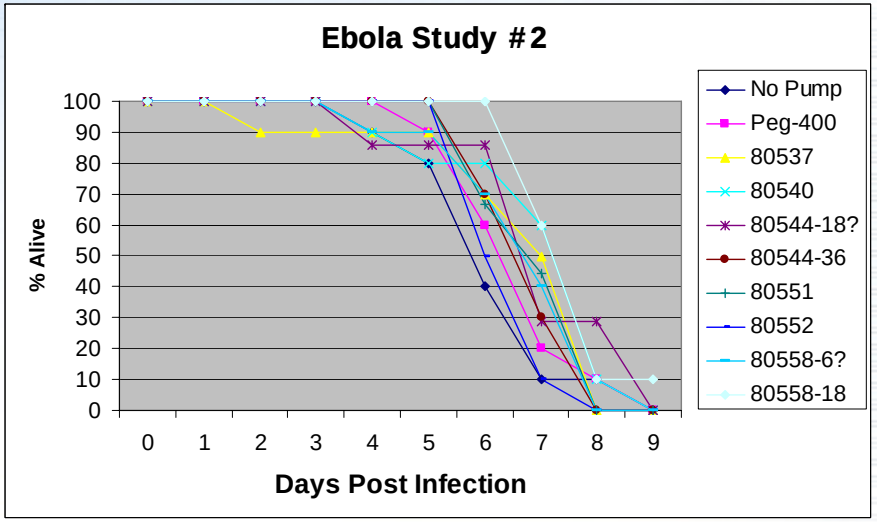
AP#	Class	Cmax (ng/ml)	Clearance In mice (ml/min/kg)	Volume of Distribution Vss (L/kg)	Halflife (hr)	IP Bioavailability
71900	**2nd SA**	**9620 ng/ml**	***37.78***	**0.71 L/kg**	**2.89 hr**	**24%**
80552	**2nd SA Novel**	**10588 ng/ml**	**20.63**	**0.48 L/kg**	**1.35 hr**	**83%**
28295	**Orphan**	**4443 ng/ml**	**14.98**	**1.88 L/kg**	**3.17 hr**	**20%**
28611	**ThPmON**	**6329 ng/ml**	**13.32**	**1.85 L/kg**	**8.81 hr**	**72%**

EBOV mouse study: Protocol

- **BALB/c Mice:**
  - female, 10-12 weeks; weight: 23-25g
- **Infection: 1000 pfu in 200µl** (ca 30,000 X LD50)
  - IP (opposite side of pump)
- **Drug delivery: Alzet pumps (IP)(25-50 mM in PEG 400)**
- **Number of mice per group: n=10**
  - 2 controls (no vehicle, vehicle)
- **Timing: Let animals recover for 2 days before infection**
  - **Day -2:** Implant Alzet osmotic pump
  - **Day 0:** EBOV Infection
- **Endpoint: Survival + weight**
  - monitor survival daily until day 8
  - monitor weight daily until day 14
  - duration of study: 30 days

Ebola mouse model studies





Inoculum: 30,000x LD_{50}, 1,000pfu

Pump: 200ul @ 1.0ul/hr for 7 days

Infection: 2 days post implant

Data: Weight loss, time of death

Inoculum: 3,000x LD_{50}, 100pfu

Pump: 200ul @ 0.5ul/hr for 14 days

Infection: 4 days post implant

Data: Weight loss, time of death, and viremia

Ongoing activities (Apath)

- Continue optimization of secondary sulfonamides
- Initiated optimization of 4-aminoquinolines
- Continue selection of compounds for mouse efficacy
- Assess spectrum of activity (other viruses, etc.)

Ongoing activities (USAMRIID)

- Other *in vitro* assays
  - Viral assays (plaque assays, RT-PCR, FACS)
  - Other cell lines (primary monocytes)
  - Other Ebola strains
  - Marburg virus
- Other animal models
  - Guinea pig
  - NHP

Ebola animal models

	Primates	Guinea pigs	Mice
Species/strain	monkeys, baboons	strain 2, 13 outbred	inbred (BALB/c, C57BL/6) outbred (CD-1)
Route	s.c., i.m., i.p. aerosol	s.c., i.m., i.p. aerosol	i.p.
Lethality	100%	100%	100%
M. T. D.	6-10 days	8-11 days	5-8 days
Target organ	liver, spleen (wide-spread infection)	liver, spleen (wide-spread infection	liver, spleen (wide-spread infection)

Summary

- Subgenomic replication represents a useful cell-based screening tool for identifying inhibitors of viruses (particularly BSL3 and 4 viruses).
- A number of lead candidates have been identified.
- Novel sulfonamide lead compounds have been identified
- Mouse efficacy studies ongoing

Acknowledgements

- **NIAID**
  - R43AI052917-01
  - R44AI052917-02
  - R01 AI066502-01 (Bioshield)
  - MRCE Development award
- **DOD**
  - DOD Contract W81XWH-05-C-0129
  - USAMRIID CRADA
    - Jason Paragas, Craig Koehler, Aura Garrison, Gene Olinger
- **Tripos, Inc.**
  - Mike Lawless, Julia Foster
- **Stephan Becker (University of Marburg)**
- **Apath**
  - Julie Dyall, Ben Buscher, Bob Roth, Urszula Slomszynska, Andy O'Guin, Gale Starkey, Jennifer Balsarotti